Biochemical Society Transactions www.biochemsoctrans.org

Calcium in the heart: when it's good, it's very very good, but when it's bad, it's horrid

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Abstract

Ca^{2+} increases in the heart control both contraction and transcription. To accommodate a short-term increased cardiovascular demand, neurohormonal modulators acting on the cardiac pacemaker and individual myocytes induce an increase in frequency and magnitude of myocyte contraction respectively. Prolonged, enhanced function results in hypertrophic growth of the heart, which is initially also associated with greater Ca^{2+} signals and cardiac contraction. As a result of disease, however, hypertrophy progresses to a decompensated state and Ca^{2+} signalling capacity and cardiac output are reduced. Here, the role that Ca^{2+} plays in the induction of hypertrophy as well as the impact that cardiac hypertrophy and failure has on Ca^{2+} fluxes will be discussed.

Introduction

The co-ordinated and efficient contraction of the four chambers of the heart is essential to ensure blood supply to the body. Moreover, it is equally critical for the heart to be able to modify its output to accommodate the changing needs of the organism from states of rest to activity. It is therefore not surprising that any factors that contribute to a loss of rhythm or contractility give rise to pathological consequences. Indeed, heart disease is a significant cause of mortality, being responsible, in 2004, for 137 700 deaths in the U.K., equating to 24% of all deaths [1]. These statistics surpass the 33 000 deaths a year from lung cancer, 16 000 deaths from colorectal cancer and 13 000 deaths from breast cancer. Decompensated cardiac hypertrophy is the most important risk factor for heart failure in humans [2]. Cardiac hypertrophy is defined by an increase in the muscle mass of the heart due to cellular enlargement without any proliferation (Figure 1) [3]. Under physiological conditions that involve a requirement for increased cardiac output, such as those experienced by performance athletes and during pregnancy, hypertrophy is an adaptive response (also known as compensated hypertrophy) [3,4]. Under pathological conditions, such as hypertension, viral infection or due to mutations in genes encoding sarcomeric proteins, hypertrophy is a maladaptive response and progresses to dilated cardiomyopathy with associated fibrosis, arrhythmias and sudden death (also known as decompensated hypertrophy) [3,4].

Key words: calcium flux, cardiac failure, cardiac myocyte, contraction, decompensated hypertrophy, heart disease.

Abbreviations used: ANF, atrial natriuretic factor; CaM, calmodulin; CaMKII, Ca^{2+}/CaM-dependent protein kinase II; CAMTA2, CaM-binding transcription activator 2; DAG, diacylglycerol; ER, endoplasmic reticulum; ET, endothelin; HDAC, histone deacetylase; InsP_3R, Ins(1,4,5)P_3 receptor; MAPK, mitogen-activated protein kinase; NFAT, nuclear factor of activated T-cells; PKB, protein kinase B; PKC, protein kinase C; RyR, ryanodine receptor; SERCA, sarcoplasmic/endoplasmic-reticulum Ca^{2+}-ATPase; SR, sarcoplasmic reticulum; TRPC, canonical transient receptor potential.

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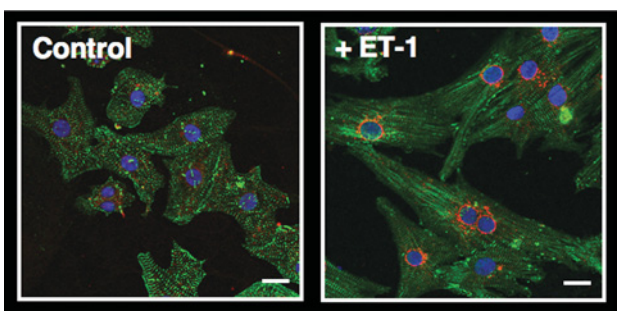
To understand the molecular mechanisms that control cardiac hypertrophy and to discover targets for pharmacological intervention, research has focused on identifying and characterizing the signalling pathways and neurohumoral factors that contribute to cellular remodelling [4–7]. This research has uncovered that hypertrophic gene transcription is induced downstream of a web of multiple interdependent signalling pathways including those regulated by MAPK (mitogen-activated protein kinase) [7,8], PI3K (phosphoinositide 3-kinase) [9,10], PKC (protein kinase C) [11,12] and Ca^{2+} [6,13–15] (Figure 2). These pathways are engaged following the activation of diverse classes of cell-surface receptors present in cardiac mycoytes, including (i) G_q-coupled receptors such as that liganded by the vasoactive peptide ET-1 (endothelin-1) [16,17], (ii) tyrosine kinase-like receptors, such as those activated by IGF (insulin-like growth factor) [18], and (iii) G_s-coupled receptors such as the β-adrenergic receptor, which plays such a profound role in cardiac biology [19,20]. Moreover, mechanical stretch and increased/modified Ca^{2+} cycling itself also activate the signalling pathways necessary for hypertrophic remodelling [21,22].

Ca^{2+} signalling to hypertrophy

As well as being directly responsible for stimulating myocyte contraction, Ca^{2+} also plays a key role in regulating the expression of hypertrophy-associated genes [6,23]. To acutely respond to greater cardiovascular demand, cardiac output is enhanced through increased Ca^{2+} fluxes. Longer-term requirements for greater cardiac output are brought about through hypertrophic growth. It is not therefore surprising that increased Ca^{2+} cycling would also be considered a trigger for the induction of hypertrophy; more work, more Ca^{2+}, more gene transcription, more heart. The reports that hypertrophy can be induced by simply enhancing Ca^{2+} fluxes, via electrical 'pacing' in both cellular and animal

Figure 1 | ET-1-induced hypertrophy in neonatal rat myocytes

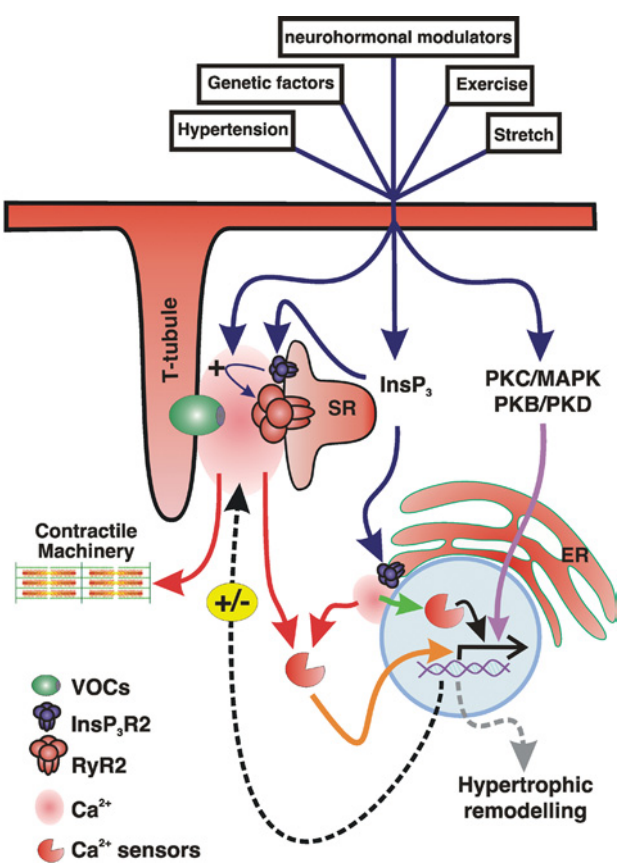
Spontaneously beating primary cultures of rat neonatal cardiac myocytes exhibit hypertrophic growth following treatment with ET-1 (100 nM) for 24 h. ET-1-treated myocytes have a greater surface area and sarcomeric organization as revealed by staining for α-actinin (green). ET-1-stimulated myocytes also express ANF, which is manifest as a perinuclear ring of immunoreactivity (red). ANF is a robust indicator of hypertrophy [57]. DAPI (4′,6-diamidino-2-phenylindole)-stained nuclei are indicated in blue. The white scale bar represents 20 μm.



models, support this concept [21,24]. Moreover, the acute enhancement of Ca^{2+} cycling experienced by myocytes during periods of increased workload or under stress is induced by the same neurohumoral factors, such as adrenalin and ET-1, that also induce hypertrophy when chronically applied [15,25]. The direct role of Ca^{2+} for the induction of hypertrophy is also suggested by evidence obtained using transgenic models. For example, transgenic overexpression of the archetypal cellular Ca^{2+} sensor, CaM (calmodulin), induces hypertrophy [26]. It is clear that Ca^{2+}-activated CaM has major signalling targets involved in the stimulation of hypertrophic gene transcription: the Ca^{2+}/CaM-activated serine/threonine phosphatase, calcineurin [also known as PP2B (protein phosphatase 2B)] [23], CAMTA2 (CaM-binding transcription activator 2) [27] and the delta isoform of CaMKII (Ca^{2+}/CaM-dependent protein kinase II) [23]. During the induction of hypertrophy, activated calcineurin dephosphorylates NFAT3 (nuclear factor of activated T-cells 3), leading to its nuclear translocation and induction of target genes bearing NFAT-binding sequences in their promoters. The role of this linear pathway in cardiac myocytes was elegantly delineated by a series of publications from the Molkentin and Olsen laboratories, which showed that hypertrophy could be induced by transgenic expression of a mutated constitutively active calcineurin or of an activated form of NFAT [28]. CaMKII signalling is linked to regulation of hypertrophic gene transcription through inducing the phosphorylation of HDACs (histone deacetylases) in the nucleus, resulting in their nuclear export and removal of their inhibitory effect on gene transcription [29]. This pathway was unravelled using transgenic expression of CaMK inhibitor peptides in the nucleus and non-phosphorylatable forms of HDAC together with the use of knockout strategies [30,31]. Moreover, in response to prohypertrophic signals, HDACs are observed to translocate out of the nucleus [32]. CAMTA2

Figure 2 | Signalling to cardiac hypertrophy

The Figure shows the intracellular pathways that are activated downstream of diverse prohypertrophic factors. Many of these stimuli activate increases in both Ca^{2+} cycling and other cellular signal-transduction cascades such as those mediated by PKC, MAPK and PKB (protein kinase B). Certain stimuli also induce increases in intracellular $InsP_3$, which subsequently promotes Ca^{2+} release through $InsP_3$Rs that are located under the plasmalemma adjacent to RyRs or around the nucleus. Ca^{2+} release through the plasmalemmal $InsP_3$Rs may contribute to their inotropic function by sensitizing RyRs located there whereas Ca^{2+} release through nuclear $InsP_3$Rs may play a role in regulating hypertrophic gene transcription. Whether these nuclear $InsP_3$Rs release Ca^{2+} directly into the nucleus or into the adjacent cytosol is not clear. Hypertrophic gene transcription is activated downstream of MAPK, PKC and PKB signalling pathways (purple arrow). Gene transcription is also induced by changes in Ca^{2+} in the cytosol (orange arrow) or increases in Ca^{2+} in the nucleus (green arrow), where it is sensed by appropriately localized Ca^{2+} sensors/transcriptional activators/co-activators. Hypertrophic remodelling is associated with increased cardiac muscle mass as well as an increase in Ca^{2+} signalling capacity (black dashed arrow). During heart failure, Ca^{2+} signalling capacity may be decreased (grey dashed arrow). VOCs, voltage-operated channels.



is the most recently described Ca^{2+}-regulator of hypertrophic gene transcription [27]. It elicits its effect by acting as a co-activator of transcription factors involved in hypertrophic gene transcription. In non-stimulated cells, it remains inactive through interaction with HDACs [27]. HDACs are also

sensitive to regulation by PKC, providing a further Ca^{2+} link to DNA modification [33].

Encoding specificity in Ca^{2+} signals

Despite the many observations that suggest the sufficiency of enhanced Ca^{2+} cycling for hypertrophic remodelling, it is not clear how this signalling role of Ca^{2+} can be encoded above the noise of the bulk changes in Ca^{2+} that occur in the myocyte during every heart beat. Indeed, since these CaM-regulated pathways detect changes in intracellular Ca^{2+} in the submicromolar range, it would be predicted that they would be activated by every Ca^{2+} transient during every heart beat, and, moreover, their activity would be further increased by inotropy. This 'Ca^{2+} only' model for the induction of hypertrophy would, however, not be advantageous for the myocyte; the array of phenotypic changes associated with hypertrophy, which include re-expression of foetally expressed genes such as ANF (atrial natriuretic factor; Figure 1), would be induced at the functional and energetic expense of the myocyte without benefit. How specificity could be imposed upon the Ca^{2+} signal is therefore not clear. At the most basic level, a mechanism would involve the simple integration of either an increase in the frequency and/or amplitude of the action potential-induced Ca^{2+} transient by the transcription machinery [34]. This would be determined by the equilibrium between the rate of activation and inactivation of the Ca^{2+} sensor as well as the rates of activation and inactivation of its target. For example, calcineurin responds to repetitive, low-amplitude Ca^{2+} transients, whereas CaMKII is activated preferentially by Ca^{2+} spikes that are transient and of high amplitude [35,36]. The equilibrium could also be shifted in favour of the activated prohypertrophic state by an increase in the diastolic Ca^{2+} level. This could occur through decreased Ca^{2+} clearance mechanisms or enhanced activation of Ca^{2+}-release channels on the SR (sarcoplasmic reticulum). The induction of hypertrophy by overexpression of canonical members of the TRP channels (TRPCs) is also suggestive of a role of diastolic Ca^{2+} in hypertrophic signalling [37].

A more attractive proposal is that the subcellular location of the Ca^{2+} signal responsible for the induction of gene transcription is different from that which stimulates contraction. Indeed, elegant work from the Bers laboratory has shown that a Ca^{2+} signal generated in a microdomain around the nucleus can promote the activation of a hypertrophy-associated gene reporter construct [32]. Moreover, nuclear localized InsP_3R [Ins(1,4,5)P_3 receptor] Ca^{2+} release channels were shown to be involved in causing the depletion of the nuclear Ca^{2+} store, which then promoted the nuclear export of HDAC (Figure 2). This situation is analogous to that observed in skeletal muscle, where Ca^{2+} is released from nuclear localized InsP_3Rs directly into the nucleoplasm, resulting in phosphorylation of the CREB (cAMP-response-element-binding protein) transcription factor [38]. Whether Ca^{2+} is released into the nucleoplasm directly or reaches there via release into the cytosol adjacent to the nucleus in cardiac myocytes remains to be determined. A further point of debate is whether this nuclear Ca^{2+} store is distinct from the SR. Although fluorescence recovery after photobleaching experiments indicate that it is contiguous with the SR [39], the requirement of the ER (endoplasmic reticulum) Ca^{2+}-binding protein calreticulin for cardiac development suggests that the ER has a specialized role in the cardiac myocyte [40]. The induction of hypertrophy as a result of enhancement of store-operated Ca^{2+} entry by expression of TRPC family members also supports a distinct signalling role for the ER in the cardiac myocyte [37].

This idea that Ca^{2+} is sufficient for hypertrophic remodelling may, however, represent an over simplification of signalling in the cardiac myocyte. The phospholamban-knockout mouse, for example, exhibits a constitutive inotropic state yet no hypertrophy, suggesting that other signals are required for the induction of hypertrophic remodelling [41]. Lessons gained over the past decade have indicated that signalling pathways are interdependent and co-regulated and are not generally simple linear pathways that link a receptor to its target. This butterfly effect therefore needs careful consideration. It is possible that evidence gained using transgenic overexpression approaches, although valuable, demonstrates only the possible functions of proteins in the hypertrophic response. It is plausible that they have been effective simply due to the ability of the overexpressed protein concerned to overcome numerous regulatory checkpoints in the cardiac myocyte signalling web. The presence of multiple regulatory sequences in the promoters of genes involved in the hypertrophic response suggests that this is the case.

Based on this concept, it is likely that hypertrophic gene transcription acts as a coincidence detector of many signalling inputs acting to continually assess both the cellular environment and activity. The signalling outputs of ET in the cardiac myocyte demonstrate this hypothesis. ET-1 is a vasoactive peptide that is found at elevated levels in the circulation of heart failure patients. Moreover, it is a potent prohypertrophic agonist. In cardiac muscle strips, ET-1 also has chronotropic, inotropic and arrhythmogenic effects. Myocytes express two receptors for ET-1, the ET_AR and the ET_BR [42]. The ET_AR is a G_q-coupled receptor, which following activation stimulates phospholipase C to cleave PtdIns(4,5)P_2 into Ins(1,4,5)P_3 and DAG (diacylglycerol) [43]. InsP_3 has recently been shown to have profound effects on Ca^{2+} handling in the cardiac myocyte; it causes inotropy and arrhythmogenic Ca^{2+} release events [25]. This effect is a little surprising since InsP_3Rs are expressed at a level almost two orders of magnitude lower than RyRs (ryanodine receptors), which are responsible for the large Ca^{2+} fluxes that induce myocyte contraction. Due to their special localizations around the nucleus and under the plasmalemma, as well as their ability to respond to stimuli other than Ca^{2+}, they may however be uniquely poised to enhance the myocyte's ability to respond and adapt to its environment [25,32] (Figure 2). DAG also has important functions in the myocyte. Its immediate target is PKC, which has possible signalling functions in regulating Ca^{2+} homoeostasis as well

as ischaemia/reperfusion injury and inducing hypertrophy [12,44]. This prohypertrophic effect of PKC has received much attention and has been shown using both transgenic and pharmacological approaches to be in part via its activation of the MAPK cascade [7,45].

Taken together, it is clear that signalling pathways that converge to induce hypertrophy are complex and require careful dissection. Due to the requirement for multiple inputs for the induction of hypertrophy, it is likely that disruption of any one of these pathways has a profound effect. Indeed, numerous gain- and loss-of-function animal models for signalling proteins have a profound effect on cardiac growth.

Impact of hypertrophy and heart failure on Ca^{2+} cycling in the cardiac myocyte

Through tipping the finely poised balance in myocyte signalling, subtle changes in Ca^{2+} cycling observed during physiology/pathophysiology may induce hypertrophy. However, hypertrophic growth, either compensated or decompensated, impacts on Ca^{2+} homoeostasis and EC (excitation–contraction) coupling. In addition to increases in cell size, adaptive hypertrophy is characterized by increased Ca^{2+}-signalling capacity. In this way, cardiac output is maximized. A recurrent theme in studies that describe the role of Ca^{2+} cycling in hypertrophied myocytes is that the amplitude of each depolarization-induced Ca^{2+} signal is increased and its rate of recovery increased [15]. This modification of the Ca^{2+} signal is brought about by changes in the expression and/or activity of Ca^{2+} handling proteins, resulting in increased SR store loading as a result of an increase in its Ca^{2+}-sequestration capacity, decreased Na^+/Ca^{2+}-exchanger activity as well as an increase in the duration of the action potential [14,15,46]. As the heart progresses to failure, however, its Ca^{2+}-signalling capacity is decreased, with Ca^{2+} release from the SR being of a lower magnitude and being less co-ordinated [47]. A significant contributor to this phenotype is a change in the ratio of the SERCA (sarcoplasmic/endoplasmic-reticulum Ca^{2+}-ATPase) pump to its negative regulator phospholamban, which changes in favour of phospholamban, resulting in less pump activity and lower SR Ca^{2+} storage [48]. The Ca^{2+} available for the pump is also sometimes decreased as a result of increased activity of the Na^+/Ca^{2+}-exchanger [49]. The SR is also depleted as a result of an increased leak through the RyR, which is brought about through the positive effect of phosphorylation and possible interaction with the immunophillin ligand FKBP12.6 (12.6 kDa FK506-binding protein) upon RyR activity [47,50]. Whether these changes in Ca^{2+} homoeostasis are a cause or consequence of disease, as well as whether they play a continued signalling role in the maintenance of the hypertrophic state, remains a matter of debate. Depending on the model being studied, both scenarios may be important. For example, recapitulation of certain Ca^{2+}-cycling defects by overexpression of mutated Ca^{2+}-handling proteins (such as calsequestrin), by themselves are sufficient to induce hypertrophy [51,52]. Interestingly, certain forms of heart failure can be rescued by enhancing Ca^{2+} signalling, for example by phospholamban deletion [48]. Furthermore, increases in Ca^{2+} transients are observed at early stages in the progression to hypertrophy [15]. The possibility that hypertrophy can itself cause changes in Ca^{2+} signals is derived from transgenic models in which hypertrophy is induced by overexpression of proteins not directly involved in Ca^{2+} homoeostasis. For example, myocytes isolated from mice transgenically overexpressing calcineurin also have altered Ca^{2+}-handling characteristics. In these animals, Ca^{2+} sequestration into the SR is increased through an up-regulation of SERCA expression and a decrease in phospholamban, both characteristic of hypertrophied myocytes [53]. Paradoxically, this enhanced Ca^{2+} signalling and associated contractility persist in individual myocytes isolated from failing hearts that actually exhibit decreased contractile properties. This discrepancy between contractility of individual myocytes and the intact heart is proposed to be due to increased fibrosis and decreased connexin-mediated connectivity between individual myocytes. These studies indicate that calcineurin signalling contributes to a positive-feedback loop that promotes the enhanced signalling capacity required for greater cardiac output as well as myocyte hypertrophy and further calcineurin activation.

These results suggest that it is likely that hypertrophy induced by genetic factors progresses differently from that induced by conditions of physiological stress (pressure overload or hormones); the requirement for an initiating Ca^{2+} signal is lost but the enhanced Ca^{2+} signals required to improve function are maintained. This enhanced Ca^{2+} signalling, however, has a negative effect on cell viability resulting in apoptosis. Myocytes lost through this process are replaced with fibroblasts, resulting in fibrosis of the heart and decreased contractility and conductivity as observed in the constitutively active calcineurin-overexpressing mouse [53]. In the case of hypertrophy induced by mechanical or hormonal stimuli, unlike that induced by genetic factors, an increase in Ca^{2+} cycling may initiate hypertrophic remodelling but as it progresses to heart failure, Ca^{2+} cycling is decreased [15,47]. The time point at which the state of the Ca^{2+} signalosome is assessed after the induction of hypertrophy is critical.

There is no doubt that Ca^{2+} homoeostasis is severely affected in heart failure models. In addition to changes in Ca^{2+}-handling protein expression and their effects on Ca^{2+} transients, the Ca^{2+} signal and its relationship with contraction are significantly affected by ultrastructural alterations observed during heart failure. At this failing stage, the t-tubular network in ventricular myocytes atrophies, resulting in a decrease in the efficiency of coupling between the action potential and Ca^{2+} release [54]. It has been proposed that as well as an increase in distance between L-type VOCCs (voltage-operated Ca^{2+} channels) and RyRs, which decreases the probability of successful CICR (Ca^{2+}-induced Ca^{2+} release), due to t-tubule loss, some RyRs are orphaned and no longer have an L-type channel partner [55]. These findings have recently been extended to the adaptive

hypertrophy model, where, although there are no orphaned RyRs, the distance between the L-type channel and the RyR is increased. Thus there is a lower probability of the small amount of Ca^{2+} entering through the L-type channels stimulating Ca^{2+} release through the RyR (decreased gain) [56].

Conclusions

It is established that Ca^{2+} signals encode multiple levels of information. The cardiac myocyte, in terms of how it responds both acutely and in the long term to its environment, is an ultimate model of how the amplitude, timing and localization of a Ca^{2+} signal can be used to specifically control cellular output and cell fate. Research to understand the interplay between these different Ca^{2+} codes and other cellular signalling pathways and how they synergize to ultimately determine cell fate will remain a focus of future investigations.

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Received 18 June 2007
doi:10.1042/BST0350957